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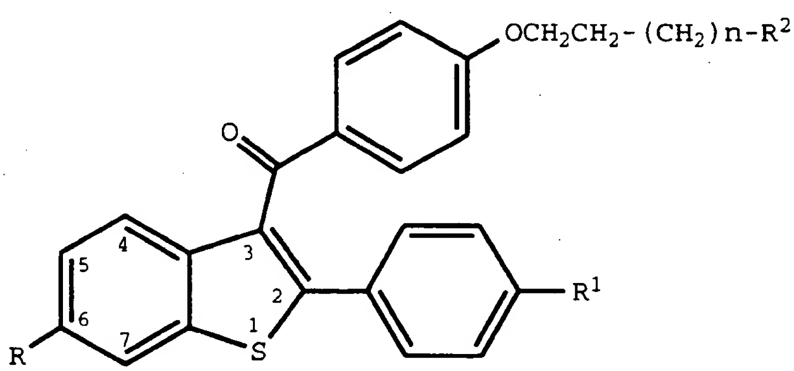
(54) Use of benzothiophenes for treatment of hypercholesterolaemia.

(57) Use of a compound of formula (I) :



(I)

wherein

n is 0, 1 or 2 ;

R is hydroxyl, methoxy, C_1-C_7 alkanoyloxy, C_3-C_7 cycloalkanoyloxy, (C_1-C_6 alkoxy)-C_1-C_7 alkanoyloxy, substituted or unsubstituted aroyloxy, or substituted or unsubstituted aryloxycarbonyloxy ;

R^1 is hydrogen, hydroxyl, chloro, bromo, methoxy, C_1-C_7 alkanoyloxy, C_3-C_7 cycloalkanoyloxy, (C_1-C_6 alkoxy)-C_1-C_7 alkanoyloxy, substituted or unsubstituted or aroyloxy, or substituted or unsubstituted aryloxycarbonyloxy ;

R^2 is a heterocyclic ring selected from the group consisting of pyrrolidino, piperidino, or hexamethyleneimino ; or a pharmaceutically acceptable salt or solvate thereof ; in the preparation of a medicament useful for lowering serum cholesterol levels in animals.

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The present invention relates to the discovery that a group of 2-phenyl-3-aroylbenzothiophenes are useful for lowering serum cholesterol.

All mammalian cells require cholesterol as a structural component of their cell membranes and for non-sterol end products. The very property, however, that makes cholesterol useful in the cell membranes, its insolubility in water, also makes it potentially lethal. When cholesterol accumulates in the wrong place, for example within the wall of an artery, it cannot be readily mobilized and its presence leads to the development of an atherosclerotic plaque. Elevated concentrations of serum cholesterol associated with low density lipoproteins (LDL'S) have been demonstrated to be a major contributing factor in the development and progression of atherosclerosis.

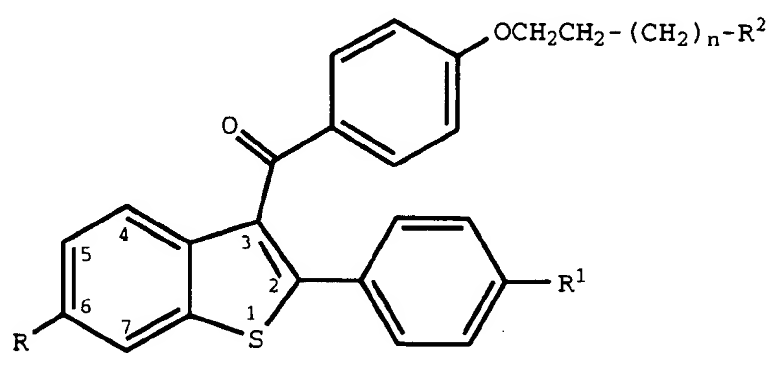
Estrogen, particularly when taken orally, lowers plasma levels of LDL and raises those of the beneficial high density lipoproteins (HDL's). Long-term estrogen therapy, however, has been implicated in a variety of disorders, including an increase in the risk of uterine cancer and possibly breast cancer, causing many women to avoid this treatment. Recently suggested therapeutic regimens, which seek to lessen the cancer risk, such as administering combinations of progestogen and estrogen, cause the patient to experience unacceptable bleeding. Furthermore, combining progesterone with estrogen seems to blunt the serum cholesterol lowering effects of estrogen. The significant undesirable effects associated with estrogen therapy support the need to develop alternative therapies for hypercholesterolemia that have the desirable effect on serum LDL but do not cause undesirable effects.

Attempts to fill this need by the use of compounds commonly known as antiestrogens, which interact with the estrogen receptor and/or bind what has been termed the antiestrogen binding site (AEBS), have had limited success, perhaps due to the fact that these compounds generally display a mixed agonist/antagonist effect and are subject to the same adverse effects associated with estrogen therapy.

The current invention provides methods for lowering serum LDL levels without the associated adverse effects of estrogen therapy, and thus provides an effective and acceptable treatment for hypercholesterolemia.

The 2-phenyl-3-aroylbenzothiophene compounds of formula I that are the active component in the methods of this invention were in a group of compounds first developed by C. David Jones and Tulio Suarez as anti-fertility agents (see U.S. Patent No. 4,133,814, issued January 9, 1979). Jones later found a group of these compounds, including the formula I compounds of this invention, to be useful for antiestrogen and antiandrogen therapy, especially in the treatment of mammary and prostatic tumors (see U.S. Patent 4,418,068, issued November 29, 1983). One of these compounds, the compound of formula I wherein n is 0, R and R^1 are hydroxyl, and R^2 is a piperidino ring, was clinically tested for a brief time for the treatment of breast cancer. That compound is called raloxifene (formerly called keoxifene).

This invention provides a new method for lowering serum cholesterol levels, comprising administering a compound of formula I



(I)

wherein

n is 0, 1 or 2;

R is hydroxyl, methoxy, C_1-C_7 alkanoyloxy, C_3-C_7 cycloalkanoyloxy, (C_1-C_6 alkoxy)-C_1-C_7 alkanoyloxy, substituted or unsubstituted aroyloxy, or substituted or unsubstituted aryloxycarbonyloxy;

R^1 is hydrogen, hydroxyl, halo, methoxy, C_1-C_7 alkanoyloxy, C_3-C_7 cycloalkanoyloxy, (C_1-C_6 alkoxy)-C_1-C_7 alkanoyloxy, substituted or unsubstituted aroyloxy, or substituted or unsubstituted aryloxycarbonyloxy;

R^2 is a heterocyclic ring selected from the group consisting of pyrrolidino, piperidino, or hexamethyleneimino; or a pharmaceutically acceptable salt or solvate thereof, to a patient in need of lower serum cholesterol levels.

This invention also provides the use of a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, in the preparation of a medicament useful for lowering serum cholesterol levels.

The general chemical terms used in the description of a compound of formula I have their usual meanings. For example, the term "alkyl" by itself or as part of another substituent means a straight or branched chain alkyl radical having the stated number of carbon atoms such as methyl, ethyl, propyl, and isopropyl and higher homologues and isomers where indicated.

The term "alkoxy" means an alkyl group having the stated number of carbon atoms linked by an oxygen atom, such as methoxy, ethoxy, propoxy, butoxy, pentyloxy, and hexyloxy and also includes branched chain structures such as, for example, isopropoxy and isobutoxy.

The term "C_1-C_7-alkanoyloxy" means a group -O- C(O)-R^a where R^a is hydrogen or C_1-C_6 alkyl and includes formyloxy, acetoxy, propanoyloxy, butanoyloxy, pentanoyloxy, hexanoyloxy, and the like and also includes branched chain isomers such as, for example, 2,2-dimethylpropanoyloxy, and 3,3-dimethylbutanoyloxy.

Analogously, the term C_4-C_7 cycloalkanoyloxy" means a group -O-C(O)-(C_3-C_6 cycloalkyl) where the C_3-C_6 alkyl group includes cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

The term "(C_1-C_6-alkoxy)-C_1-C_7-alkanoyloxy" means a group -O-C(O)-R^b-O-(C_1-C_6 alkyl) where R^b is a bond (C_1-C_6 alkoxycarbonyloxy) or C_1-C_6 alkanediyl and includes, for example, methoxycarbonyloxy, ethoxycarbonyloxy, propoxycarbonyloxy, butoxycarbonyloxy, methoxyacetoxy, methoxypropanoyloxy, methoxybutanoyloxy, methoxypentanoyloxy, methoxyhexanoyloxy, ethoxyacetoxy, ethoxypropanoyloxy, ethoxybutanoyloxy, ethoxypentanoyloxy, ethoxyhexanoyloxy, propoxyacetoxy, propoxypropanoyloxy, propoxybutanoyloxy, and the like.

The term "unsubstituted or substituted aroyloxy" means a group -O-C(O)-aryl where aryl is a phenyl, naphthyl, thienyl or furyl group that is, as to each group, unsubstituted or monosubstituted with a hydroxyl, halo, C_1-C_3 alkyl, or C_1-C_3 alkoxy substituent.

The term "unsubstituted or substituted aryloxycarbonyloxy" means a group -O-C(O)-O-aryl where aryl is a phenyl, naphthyl, thienyl or furyl group that is, as to each group, unsubstituted or monosubstituted with a hydroxyl, halo, C_1-C_3 alkyl or C_1-C_3 alkoxy substituent.

The term "halo" means chloro, fluoro, bromo or iodo.

The current invention concerns the discovery that a select group of 2-phenyl-3-aroylbenzothiophenes, the compounds of formula I, are useful for lowering serum cholesterol levels. The methods of treatment provided by this invention comprise administering a compound of formula I, or a pharmaceutically acceptable acid addition salt or solvate thereof, to a patient in need of lower serum cholesterol levels. The present method includes both medical therapeutic and/or prophylactic treatment, as appropriate. Generally, the compound is formulated with common excipients, diluents or carriers, and compressed into tablets, or formulated as elixirs or solutions for convenient oral administration, or administered by the intramuscular or intravenous routes. The compounds can be administered transdermally, and are well suited to formulation as sustained release dosage forms and the like.

The benzothiophenes of formula I lower serum cholesterol levels in animals and should be particularly useful for lowering cholesterol levels in humans. Excess serum cholesterol may result from a variety of conditions and disorders, including a lack of endogenous estrogen such as occurs in women following cessation of menstruation due to natural, surgical, or other processes, and patients having gonadal dysgenesis.

The benzothiophenes of formula I are a series of nonsteroidal compounds that exhibit high affinity for conventional estrogen receptors in primary sex target tissues. However, they elicit minimal estrogenic responses in those tissues, and actually serve as potent antagonists of natural estrogens such as estradiol. In contrast to other structurally distinct antiestrogen compounds, the benzothiophenes of formula I are able to antagonize classical estrogenic responses in primary sex target tissues while eliciting an estrogenic response on serum cholesterol levels. This dichotomy indicates selective agonist/antagonist actions on specific target cells which are highly desirable in treating hypercholesterolemia. Thus, the current invention provides a method of lowering serum cholesterol levels, comprising administering to a patient an amount of a compound of formula I that lowers serum LDL levels, but does not significantly affect the primary sex target tissues. This combination of features allows for long-term treatment of the chronic ailment with little risk of developing the undesirable effects of customary estrogen replacement therapy.

The biological action of the benzothiophenes of formula I is complex and may be unrelated to the detectable presence of the parent compound in the blood. Following oral administration of a preferred benzothiophene of this invention, raloxifene (administered as the hydrochloride), to human subjects in the clinic, the parent compound was not detected in the serum of those subjects. It was determined that following oral ad-

ministration, the compound was extensively conjugated to the glucuronidated form. Although no biological endpoints were measured in the human recipients, there was concern that the compound was not bioavailable.

Experiments were undertaken to address the bioavailability issue in laboratory animals where biological activity could be assessed. The animal studies indicated that raloxifene was maximally active in inhibiting both uterine uptake of tritiated-estradiol and the normal uterotrophic response to estradiol even under conditions where raloxifene was extensively conjugated in the plasma of the animals. Moreover, the conjugate, isolated from the urine of human subjects treated with raloxifene, displayed significant antiestrogenic/antiuterotrophic activity when administered intravenously to rats, and inhibited the interaction of tritiated-estradiol with rat uterine estrogen receptors in a manner similar to the parent compound at temperatures approaching physiologic conditions. Since these compounds did not bind at 4°C, these studies suggested the conjugated compound may have been converted to the parent form at the site of action, presumably by the action of β-glucuronidase. Such conversion may contribute to the activity of the compound. β-Glucuronidase is fairly ubiquitous and would presumably be available for converting the conjugated compound to the parental form if required for activity. Therefore, conjugation of the benzothiophenes of formula I is not considered to be necessarily detrimental to their bioavailability.

Thus, the method of treatment provided by this invention is practiced by administering to a patient an amount of a compound of formula I, or a pharmaceutically acceptable salt or solvate thereof, that is effective to lower serum cholesterol levels. A particular benefit of this method is that it avoids potentially harmful and unacceptable estrogenic side effects. The present method includes both medical therapeutic and/or prophylactic treatment, as appropriate.

The method also includes the administration of a compound of formula I and estrogen, either independently or in combination. The term estrogen as used herein refers to any compound which approximates the spectrum of activities of the naturally acting molecule which is commonly believed to be 17β-estradiol. Examples of such compounds include estriol, estrone, ethynyl estradiol, Premarin (a commercial preparation of conjugated estrogens isolated from natural sources - Ayerst), and the like. Again, due to the selective agonist/antagonist properties of the compounds of formula I, this combination provides the benefits of estrogen therapy without the concomitant adverse effects associated with estrogen therapy alone.

Preferred methods of this invention comprise the use of compounds of formula I wherein n is O; and R and R^1 are independently hydroxyl, C_1-C_7 alkanoyloxy, (C_1-C_6 alkoxy)-C_1-C_7 alkanoyloxy, unsubstituted or substituted benzoyloxy or unsubstituted or substituted phenoxycarbonyloxy. Further preferred methods include the use of formula I compounds wherein R and R^1 are the same. Certain R^2 groups also demonstrate preferable characteristics when used in the methods of this invention. For example, preferred methods of this invention include the use of formula I compounds wherein R^2 is piperidino or pyrrolidino. Especially preferred are those compounds where R^2 is piperidino or pyrrolidino and R and R^1 are hydroxyl. A most preferred formula I compound is raloxifene.

The formula I compounds and their pharmaceutically acceptable salts can be made according to established procedures, such as those detailed in U.S. Patent Nos. 4,133,814 and 4,418,068.

The formula I compounds form acid and base addition salts with a wide variety of organic and inorganic acids and bases and include the physiologically acceptable salts which are often used in pharmaceutical chemistry. Such salts are also part of this invention. Typical inorganic acids used to form such salts include hydrochloric, hydrobromic, hydroiodic, nitric, sulfuric, phosphoric, hypophosphoric and the like. Salts derived from organic acids, such as aliphatic mono and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxyalkanoic and hydroxyalkandioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, may also be used. Typical salts include acetate, phenylacetate, trifluoroacetate, acrylate, ascorbate, benzoate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, bromide, isobutyrate, phenylbutyrate, β-hydroxybutyrate, butyne-1,4-dioate, hexyne-1,4-dioate, caprate, caprylate, chloride, cinnamate, citrate, formate, fumarate, glycollate, heptanoate, hippurate, lactate, malate, maleate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, isonicotinate, nitrate, oxalate, phthalate, teraphthalate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, propiolate, propionate, phenylpropionate, salicylate, sebacate, succinate, suberate, sulfate, bisulfate, pyrosulfate, sulfite, bisulfite, sulfonate, benzene-sulfonate, p-bromophenylsulfonate, chlorobenzenesulfonate, ethanesulfonate, 2-hydroxyethanesulfonate, methanesulfonate, naphthalene-l-sulfonate, naphthalene-2-sulfonate, p-toluenesulfonate, xylenesulfonate, tartarate, and the like.

In addition, some of the formula I compounds may form solvates with water or organic solvents such as ethanol. These solvates are also contemplated for use in the methods of this invention.

Bases commonly used for formation of salts include ammonium hydroxide and alkali and alkaline earth metal hydroxides, carbonates and bicarbonates, as well as aliphatic and aromatic amines, aliphatic diamines and hydroxy-alkylamines. Bases especially useful in the preparation of addition salts include ammonium hy-

droxide, potassium carbonate, sodium bicarbonate, calcium hydroxide, methylamine, diethylamine, ethylene diamine, cyclohexylamine and ethanolamine.

The pharmaceutically acceptable salts generally have enhanced solubility characteristics compared to the compound from which they are derived, and thus are often more amenable to formulation as liquids or emulsions.

In another aspect, this invention provides a process for the preparation of a pharmaceutical composition which comprises admixing a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, prepared in known manner with suitable inert carriers and finishing the mixture to a pharmaceutical formulation suitable for use in lowering serum cholesterol levels.

This invention also contemplates a pharmaceutical formulation adapted for lowering cholesterol levels, comprising as an active ingredient a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof.

Pharmaceutical formulations can be prepared by procedures known in the art. For example, the compounds, either alone or in combination with estrogen, can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, suspensions, powders, and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include the following: fillers and extenders such as starch, sugars, mannitol, and silicic derivatives; binding agents such as carboxymethyl cellulose and other cellulose derivatives, alginates, gelatin, and polyvinylpyrrolidone; moisturizing agents such as glycerol; disintegrating agents such as agaragar, calcium carbonate, and sodium bicarbonate; agents for retarding dissolution such as paraffin; resorption accelerators such as quaternary ammonium compounds; surface active agents such as cetyl alcohol, glycerol monostearate; adsorptive carriers such as kaolin and bentonite; and lubricants such as talc, calcium and magnesium stearate, and solid polyethyl glycols.

The compounds, either alone or in combination with estrogen, can also be formulated as elixirs or solutions for convenient oral administration or as solutions appropriate for parenteral administration, for instance by intramuscular, subcutaneous or intravenous routes. Additionally, the compounds, either alone or in combination with estrogen, can be formulated as sustained release dosage forms and the like. The formulations can be so constituted that they release the active ingredient only or preferably in a particular part of the intestinal tract, possibly over a period of time. The coatings, envelopes, and protective matrices may be made, for example, from polymeric substances or waxes.

The particular dosage of a compound of formula I required to lower serum cholesterol levels according to this invention will depend upon the severity of the condition, the route of administration, and related factors. Generally, accepted and effective daily doses will be from about 0.1 to about 1000 mg, and more typically from about 50 to about 200 mg. Such dosages will be administered to a patient in need of treatment from once to about three times each day, or more often as needed to effectively lower serum cholesterol levels. Generally, accepted and effective daily doses of estrogen will be from about 0.01 to about 4.0 mg, and more typically from about 0.1 to about 2.0 mg. Such doses are administered to a patient in need of treatment from once to about three times a day, or more often as needed.

The method of the present invention is useful in men, as well as women. The substantial absence of estrogenic response should allow men to benefit from this invention without evidencing the feminizing response of estrogen or estrogen agonists such as gynecomastia. The method is especially useful in women, preferably estrogen-deficient women. The estrogen deficiency could occur naturally, such as post-menopausal, or surgically. Patients undergoing or having undergone long-term administration of corticosteroids and those having gonadal dysgenesis may also employ the method of the present invention.

It is usually preferred to administer a compound of formula I in the form of an acid addition salt, as is customary in the administration of pharmaceuticals bearing a basic group, such as the piperidino ring. It is also advantageous to administer such a compound by the oral route to an aging human (e.g. a post-menopausal female).

The following are examples of oral dosage forms useful in this invention ("Active ingredient" means a compound of formula I).

Capsules Hard gelatin capsules are prepared using one of Formulations 1-5:

Formulation 1:

Ingredient	Quantity (mg/capsule)
Active ingredient	0.1 - 1000
Starch, NF	0 - 650
Starch flowable powder	0 - 650
Silicone fluid 350 centistokes	0 - 15

The ingredients are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules.

Formulation 2:

Ingredient	Quantity (mg/capsule)
Raloxifene hydrochloride	1
Starch, NF	112
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 3:

Ingredient	Quantity (mg/capsule)
Raloxifene hydrochloride	5
Starch, NF	108
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 4:

Ingredient	Quantity (mg/capsule)
Raloxifene hydrochloride	10
Starch, NF	103
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 5:

Ingredient	Quantity (mg/capsule)
Raloxifene hydrochloride	50
Starch, NF	150
Starch flowable powder	397
Silicone fluid 350 centistokes	3.0

Tablets

Tablets are prepared using Formulation 6 or 7:

Formulation 6:

Ingredient	Quantity (mg/tablet)
Active ingredient	0.1 - 1000
Cellulose, microcrystalline	0 - 650
Silicon dioxide, fumed	0 - 650
Stearate acid	0 - 15

The components are blended and compressed to form tablets.

Alternatively, tablets each containing 0.1 - 1000 mg of active ingredient are made up as follows:

Formulation 7:

Ingredient	Quantity (mg/tablets)
Raloxifene hydrochloride	0.1 - 1000
Starch	45
Cellulose, microcrystalline	35
Polyvinylpyrrolidone (as 10% solution in water)	4
Sodium carboxymethyl cellulose	4.5
Magnesium stearate	0.5
Talc	1

The active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°-60° C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 60 U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets.

Suspensions

Suspensions containing 0.1 - 1000 mg of medicament per 5 mL dose are made as follows:

Formulation 8:

Ingredient	Quantity (amount/5mL)
Active ingredient	0.1 - 1000 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 mg
Benzoic acid solution	0.10 mL
Flavor	q.v.
Color	q.v.
Purified water	q.s. to 5 mL

The medicament is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor, and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume.

Illustrative compounds of formula I used in the methods of this invention are shown in Table 1:

Table 1

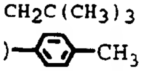
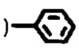
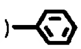
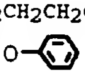
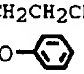
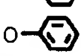
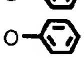
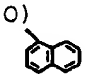
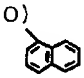
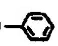
Example No.	n	R	R^1	R^2	Salt
1	0	-OC (O)	-OC (O)	piperidino	
2	0	-OC (O)	-OC (O)	piperidino	HCl
3	0	-OC (O)	-OC (O)	piperidino	
4	0	-OC (O)	-OC (O)	piperidino	HCl
5	0	$-OC(O)CH_2CH_2CH_3$	$-OC(O)CH_2CH_2CH_3$	piperidino	
6	0	$-OC(O)CH_2CH_2CH_3$	$-OC(O)CH_2CH_2CH_3$	piperidino	HCl
7	0	$-OC(O)C(CH_3)_3$	$-OC(O)C(CH_3)_3$	piperidino	
8	0	$-OC(O)C(CH_3)_3$	$-OC(O)C(CH_3)_3$	piperidino	HCl
9	0	$-OC(O)CH_2C(CH_3)_3$	$-OC(O)CH_2C(CH_3)_3$	piperidino	
10	0	$-OC(O)CH_2C(CH_3)_3$	$-OC(O)CH_2C(CH_3)_3$	piperidino	HCl
11	0	-OC (O)	-OC (O)	piperidino	
12	0	-OC (O)	-OC (O)	piperidino	HCl
13	0	-OC (O)	-OC (O)	piperidino	
14	0	$-OC(O)OCH_2CH_2CH_2CH_3$	$OC(O)OCH_2CH_2CH_2CH_3$	piperidino	
15	0	$-OC(O)OCH_2CH_2CH_2CH_3$	$OC(O)OCH_2CH_2CH_2CH_3$	piperidino	HCl
16	0	-OC (O) O	-OC (O) O	piperidino	
17	0	-OC (O) O	-OC (O) O	piperidino	HCl
18	0	-O-C (O)	-O-C (O)	piperidino	
19	0	$-OC(O)CH_2CH_2OCH_3$	$-OC(O)CH_2CH_2OCH_3$	piperidino	
20	0	$-OC(O)CH_2CH_2OCH_3$	$-OC(O)CH_2CH_2OCH_3$	piperidino	HCl
21	0	-OH	-OH	piperidino	HCl

Table 1 (cont.)

Example No.	n	R	R^1	R^2	Salt
22	0	-OH	-OH	piperidino	
23	1	-OH	-OH	piperidino	HCl
24	0	-OH	-OH	pyrrolidino	
25	0	-OH	-OH	pyrrolidino	HCl
26	0	-OH	-OH	hexamethyleneimino	HCl
27	0	$-OCH_3$	$-OCH_3$	piperidino	HCl
28	0	$-OC(O)CH_3$	$-OC(O)CH_3$	piperidino	HCl
29	0	$-OCH_3$	H	pyrrolidono	citrate
30	0	$-OCH_3$	$-OCH_3$	pyrrolidino	citrate
31	0	-OC(O)- 	-OC(O)- 	piperidino	HCl
32	0	-OH	Cl	piperidino	HCl
33	0	-OH	F	piperidino	HCl

The following test examples illustrate the methods of this invention.

Test Procedure

In the examples illustrating the methods, a post-menopausal model was used in which effects of different treatments upon circulating lipids were determined.

Seventy-five day old female Sprague Dawley rats (weight range of 200 to 225 g) were obtained from Charles River Laboratories (Portage, MI). The animals were either bilaterally ovariectomized (OVX) or exposed to a Sham surgical procedure at Charles River Laboratories, and then shipped after one week. Upon arrival, they were housed in metal hanging cages in groups of 3 or 4 per cage and had *ad libitum* access to food (calcium content approximately 0.5%) and water for one week. Room temperature was maintained at 22.2° ± 1.7° C with a minimum relative humidity of 40%. The photoperiod in the room was 12 hours light and 12 hours dark.

Dosing Regimen/TissueCollection.

After a one week acclimation period (therefore, two weeks post-OVX) daily dosing with test compound was initiated. All compounds were administered orally at 1 ml/kg body weight unless otherwise stated. 17β-Estradiol was administered subcutaneously in a 20% polyethylene glycol vehicle: 17α-ethynyl estradiol and the test compound were given orally, unless otherwise stated, as a suspension in 1% carboxymethylcellulose or 20% cyclodextrin. Animals were dosed daily for 4 days. Following the dosing regimen animals were weighed and anesthetized with a ketamine: Xylazine (2:1, V:V) mixture, and a blood sample was collected by cardiac puncture. Each animal was then sacrificed by asphyxiation with CO_2; the uterus was removed through a mid-line incision and a wet weight was determined.

Cholestrol Analysis.

Blood samples were allowed to clot at room temperature for 2 hrs, and serum was obtained following centrifugation for 10 min at 3000 rpm. Serum cholesterol was determined using a Boehringer Mannheim Diagnostics high performance cholesterol assay. Briefly, the cholesterol was oxidized to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide was then reacted with phenol and 4-aminophenazone in the pres-

ence of peroxidase to produce a p-quinone imine dye, which was read spectrophotemetrically at 500 nm. Cholesterol concentration was then calculated against a standard curve. The entire assay was automated using a Biomek Automated Workstation.

Uterine Eosinophil Peroxidase (EPO) Assay.

Uteri were kept at 4° C until time of enzymatic analysis. The uteri were then homogenized in 50 volumes of 50 mM Tris buffer (pH - 8.0) containing 0.005% Triton X-100. Upon addition of 0.01% hydrogen peroxide and 10 mM o-phenylenediamine (final concentrations) in Tris buffer, increase in absorbance was monitored for one minute at 450 nm. The presence of eosinophils in the uterus is an indication of estrogenic activity of a compound. The maximal velocity of a 15 second interval was determined over the initial, linear portion of the reaction curve.

Sources of Compounds:

17β-estradiol, 17α-ethynyl estradiol and tamoxifen were obtained from Sigma Chemical Co., St. Louis, MO.

Experimental Groups:

All experimental groups were comprised of five or six animals.

INFLUENCE OF RALOXIFENE ON SERUM CHOLESTEROL

The results of control treatments are presented in Table 1. In summary, ovariectomy of the rats caused an increase in serum cholesterol as compared to intact vehicle treated controls. Estrogen, administered in the orally active form of ethynyl estradiol (EE_2), caused a decrease in serum cholesterol in a dose dependent manner, but it also exerted a stimulatory action on the uterus resulting in uterine weights approaching that of an intact rat when administered at 100μg/kg/day. Results are reported as the mean of measurements from 5 to 6 rats ± the standard error of the mean.

In these studies, raloxifene, administered as the hydrochloride salt, also caused a serum cholesterol decrease in a dose dependent manner; however, only minimal increase of uterine weight over the ovariectomized controls was present in these animals. The effects of raloxifene are represented in Table 2. Accordingly, each point reflects the responses of 5 to 6 rats and depicts a typical dose response curve for raloxifene in this model. Results are reported as the mean ± the standard error of the mean.

TABLE 1

	Serum Cholesterol (mg/dL)	Uterine Weight Ratio (mg uterus/g body weight)	EPO Activity (m OD/min)
Ovariectomy control (0.3 mL CMC)	81.3 ± 13.4	0.48 ± 0.04	5 ± 2
Intact control (0.3 mL CMC)	72.6 ± 14.6	1.70 ± 0.12	216 ± 32
EE_2 0.1 mg/kg	46.5 ± 5.8	1.45 ± 0.08	366 ± 17

TABLE 2

	Serum Cholesterol (mg/dL)	Uterine Weight Ratio (mg uterus/g body weight)	EPO Activity (m OD/min)
Ovariectomy control (0.3 mL CMC)	87.5 ± 8.1	0.45 ± 0.02	4.8 ± 1.6
EE_2 0.1 mg/kg	8.1 ± 1.6	1.01 ± 0.03	295.1 ± 32.5
raloxifene 0.01 mg/kg	57.5 ± 6.9	0.54 ± 0.04	6.6 ± 1.4
raloxifene 0.10 mg/kg	35.3 ± 3.2	0.54 ± 0.04	5.8 ± 0.6
raloxifene 1.00 mg/kg	31.6 ± 3.4	0.56 ± 0.04	7.2 ± 2.0

Raloxifene was administered, as the hydrochloride salt, alone or in combination with 17β-estradiol. Rats treated with raloxifene alone had uterine weights which were marginally higher than the ovariectomized controls and much less than those of 17β-estradiol treated rats, which approached those of the intact controls. Conversely, raloxifene treatment substantially reduced serum cholesterol in ovariectomized rats. When given in combination with 17β-estradiol, the 17β-estradiol did not appreciably reduce the effects of raloxifene on serum cholesterol. The results are shown in Table 3.

TABLE 3

Experiment A	Serum Cholesterol (mg/dL)	Uterine Weight Ratio (mg uterus/g body weight)	EPO Activity (m OD/min)
Ovariectomy control (0.3 mL CMC)	47.8 ± 8.2	0.62 ± 0.04	8 ± 2
Intact control (0.3 mL CMC)	48.6 ± 7.3	2.25 ± 0.14	245 ± 27
17β-estradiol 0.1 mg/kg	39.6 ± 4.6	1.41 ± 0.04	403 ± 55
17β-estradiol 0.1 mg/kg + raloxifene 10 mg/kg	19.3 ± 4.3	0.99 ± 0.04	83 ± 31
raloxifene 10 mg/kg	25.6 ± 7.1	0.68 ± 0.04	2 ± 1

The ability of raloxifene to lower serum cholesterol was compared to that of tamoxifen (SIGMA, St. Louis, Mo). Tamoxifen, a well known antiestrogen currently used in the treatment of certain cancers, has been shown to lower serum cholesterol (see for example, Love, R., et al., J. Nat. Can. Inst., 82, 1327-1332 (1990). A range of doses of raloxifene and tamoxifen was administered orally to ovariectomized rats as in the previous evaluation. Although both agents displayed the ability to lower serum cholesterol while evoking only modest uterotrophic activity, as identified by gains in uterine weight, a comparison of several histological parameters demonstrated a marked difference between the rats treated with these agents. The data are set forth in Tables 4 and 5, infra.

Increases in epithelial height are a sign of estrogenicity of therapeutic agents and may be associated with increased incidence of uterine cancer. When raloxifene was administered as described supra, there was no statistically measurable increase in epithelial height over the ovariectomized controls. This result was in contrast to the results seen with tamoxifen and estrogen. At all doses given, tamoxifen increased epithelial height equal to that of an intact rat. Estradiol treatment increased epithelial height to a thickness greater than intact rats.

Estrogenicity was also assessed by evaluating the adverse response of eosinophil infiltration into the uterus. Raloxifene did not cause any increase in the number of eosinophils observed in the stromal layer of ovariectomized rats while tamoxifen caused a substantial increase in the response. Estradiol, as expected, caused

a large increase in eosinophil infiltration.

Little or no difference was detectable between raloxifene and tamoxifen effects on thickness of the stroma and myometrium. Both agents caused an increase in these measurements that was much less than the effect of estrogen.

A total score of estrogenicity, which was a compilation of all four parameters, showed that raloxifene was substantially less estrogenic than tamoxifen.

TABLE 4

	Serum Cholesterol mg/dL	Uterine Weight Ratio (mg uterus/g body weight)	EPO Activity (m OD/min)
Ovariectomy control (0.3 mL CMC)	61.4 ± 3.6	0.42 ± 0.05	4.3 ± 0.2
EE_2 100 µg/kg	9.1 ± 2.0	0.93 ± 0.08	155.6 ± 45.4
raloxifene 1 mg/kg	35.8 ± 4.1	0.54 ± 0.03	5.0 ± 0.6
tamoxifen 1 mg/kg	36.5 ± 2.8	0.76 ± 0.04	130.4 ± 31.4

TABLE 5

	Epithelial Height	Stromal Eosinophils	Myometrial Thickness	Stromal Expansion
Ovariectomy control (0.3 mL CMC)	1.24	1.00	4.42	10.83
Intact control (0.3 mL CMC)	2.71	4.17	8.67	20.67
EE_2 100 µg/kg	3.42	5.17	8.92	21.17
raloxifene 1 mg/kg (subcutaneously)	1.67	1.17	5.42	14.00
tamoxifen 1 mg/kg (subcutaneously)	2.58	2.83	5.50	14.17

Other compounds of formula I were administered orally in the rat assay described supra. Table 6 reports the effect of a 1 mg/kg dose of several compounds in terms of a percent decrease of serum cholesterol, percent uterine weight increase and EPO activity, and Table 7 reports the effects of varying doses of compounds 32 and 33 in these assays.

TABLE 6

Compound of Example Number	% Decrease of Serum Cholesterol[a]	% Uterine Weight Gain[b]	EPO Activity (m OD/min)[c]
8	64.4	49.2	9.1
10	71.9	45.3	4.3
12	75.6	41.6	4.6
13	69.7	35.9	5.5
16	80.2	43.9	3.1
17	55.2	7.1	8.2
22	75.6	38.1	4.6
23	49.5	87.5	16.3
24	73.0	48.9	9.5
25	81.6	10.6	16.4
26	64.1	53.8	5.6
27	32.9	58.0	-
30	15.6	4.5	1.9
31	68.0	38.6	5.2

[a] Percent decrease of serum cholesterol equals (serum cholesterol of treated OVX animals minus serum cholesterol of untreated OVX animals) divided by (serum cholesterol of OVX animals) multiplied by 100%.

[b] Percent uterine weight gain equals (uterine weight of treated OVX animals minus uterine weight of OVX animals) divided by (uterine weight of OVX animals) multiplied by 100%.

[c] Vmax for eosinophil peroxidase activity.

TABLE 7

Activity of Compound Number 32

Compound	Dose (mg/kg)	% Decrease of Serum Cholesterol[a]	% Uterine Weight Increase over OVX[b]	Uterine EPO Activity (m OD/min)[c]
EE2	0.1	89.7	164.1	144.5
32	0.1	32.3	56.6	8
32	1	69.9	46.3	8.8
32	10	65.0	39.0	6
33	0.1	33.8	10.9	3.5
33	1	46.0	37.7	11.4
33	10	41.3	66.8	8.9

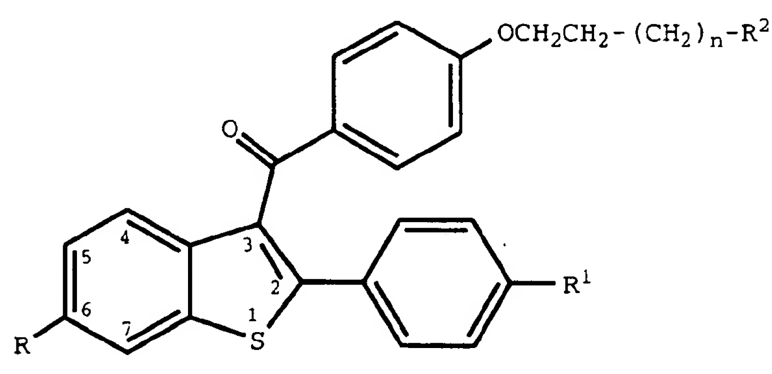
[a] Percent decrease of serum cholesterol equals (serum cholesterol of treated OVX animals minus serum cholesterol of untreated OVX animals) divided by (serum cholesterol of OVX animals) multiplied by 100%.

[b] Percent uterine weight gain equals (uterine weight of treated OVX animals minus uterine weight of OVX animals) divided by (uterine weight of OVX animals) multiplied by 100%.

[c] Vmax for eosinophil peroxidase activity.

Claims

1. The use of a compound of formula (I):



(I)

wherein

n is 0, 1 or 2;

R is hydroxyl, methoxy, C_1-C_7 alkanoyloxy, C_3-C_7 cycloalkanoyloxy, (C_1-C_6 alkoxy)-C_1-C_7 alkanoyloxy, substituted or unsubstituted aroyloxy, or substituted or unsubstituted aryloxycarbonyloxy;

R^1 is hydrogen, hydroxyl, halo, methoxy, C_1-C_7 alkanoyloxy, C_3-C_7 cycloalkanoyloxy, (C_1-C_6 alkoxy)-C_1-C_7 alkanoyloxy, substituted or unsubstituted aroyloxy, or substituted or unsubstituted aryloxycarbonyloxy;

R^2 is a heterocyclic ring selected from the group consisting of pyrrolidino, piperidino, or hexamethyleneimino;

or a pharmaceutically acceptable salt or solvate thereof, in the preparation of a medicament useful for lowering serum cholesterol levels.

2. The use of raloxifene, or a pharmaceutically acceptable salt or solvate thereof, in the preparation of a medicament useful for lowering serum cholesterol levels in a human.

3. The use of raloxifene hydrochloride in the preparation of a medicament useful for lowering serum cholesterol levels in a human.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 93 31 0438

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
T	THE JOURNAL OF CLINICAL INVESTIGATION vol. 93, no. 1 , January 1994 pages 63 - 69 BLACK, L.J. ET AL 'RALOXIFENE (LY139481) PREVENTS BONE LOSS AND REDUCES SERUM CHOLESTEROL WITHOUT CAUSING UTERINE HYPERTROPHY IN OVARECTOMIZED RATS' * the whole document *	1-3	A61K31/40 A61K31/445 A61K31/55
A	BIOCHIMICA ET BIOPHYSICA ACTA vol. 972, no. 2 , 1988 pages 167 - 178 CYPRIANI, B. ET AL 'EFFECT OF ESTRADIOL AND ANTIESTROGENS ON CHOLESTEROL BIOSYNTHESIS IN HORMONE-DEPENDENT AND -INDEPENDENT BREAST CANCER CELL LINES' * the whole document *	1-3	
A	BIOCHEMICAL PHARMACOLOGY vol. 37, no. 2 , 1988 pages 319 - 326 WEINSTEIN, I. ET AL 'EFFECTS OF THE ANTIOESTROGEN LY 117018 ON THE MODULATION BY ESTRADIOL OF THE METABOLISM OF[1-14C]OLEIC ACID BY PERFUSED LIVERS FROM NORMAL AND OVARECTOMIZED RATS' * the whole document *	1-3	TECHNICAL FIELDS SEARCHED (Int.Cl.5) A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	15 March 1994	Mair, J

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document